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(FILE 'HOME' ENTERED AT 17:06:01 ON 12 DEC 2001)

FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, BIOSIS, MEDICONF'
ENTERED AT 17:07:05 ON 12 DEC 2001

```
L1        22660 S MESENCEPHALON
L2        11586 S L1 AND (NEURON OR NEURAL)
L3         4156 S L2 AND (IMMORTAL? OR TRANS?)
L4         2023 S L3 AND (DOPAMINE? OR GABA?)
L5          279 S L4 AND (GDNF OR CTNF OR IGF-I OR BDNF OR EGF OR PDGF)
L6            1 S L5 AND V-MYC
L7            0 S L1 AND (V-MVC OR TERTACYCLINE)
L8            6 S L1 AND (V-MVC OR TETRACYCLINE)
L9            8 S L1 AND (V-MYC OR TETRACYCLINE)
L10           5 DUP REM L9 (3 DUPLICATES REMOVED)
L11          41 S L5 AND SERUM?
L12          16 DUP REM L11 (25 DUPLICATES REMOVED)
L13          16 SORT L12 PY
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FILE 'STNGUIDE' ENTERED AT 17:35:50 ON 12 DEC 2001

FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, BIOSIS, MEDICONF'
ENTERED AT 17:39:01 ON 12 DEC 2001

=> d an ti so au ab l13 6

L13 ANSWER 6 OF 16 MEDLINE
AN 96429942 MEDLINE
TI Evidence for a novel neurotrophic factor for **dopaminergic neurons** secreted from mesencephalic glial cell lines.
SO JOURNAL OF NEUROSCIENCE RESEARCH, (1996 Mar 1) 43 (5) 576-86.
Journal code: KAC; 7600111. ISSN: 0360-4012.
AU Engele J; Rieck H; Choi-Lundberg D; Bohn M C
AB Our previous studies have shown that primary mesencephalic glia secrete factors that promote **dopaminergic** cell survival and differentiation in vitro. To obtain enough starting material to identify the neurotrophic activity, embryonic day (E)14.5 rat mesencephalic glia were stimulated with acidic fibroblast growth factor to increase number of cells. These cells were replated in the absence of **neurons** and **immortalized** by **transfection** with the SV 40 large T-antigen. Clonal cell lines were established and characterized for immunoreactivity (IR) to various glial and non-glial markers. Media conditioned by these cell lines were tested for survival-promoting effects on **dopaminergic neurons** in **serum**-free cultures of the dissociated E14.5 rat **mesencephalon**. All cell lines expressed IR for the astrocytic marker, GFAP, the oligodendroglial marker, CNP, and for A2B5, a marker for O-2A progenitor cells, but were negative for the neuronal marker, microtubule associated protein-2, and the fibroblast marker, fibronectin. Moreover, treatment of **serum** -free cultures of the dissociated E14.5 **mesencephalon** with glial cell line-CM conditioned medium (CM) delayed **dopaminergic** cell death in a dose-dependent manner, resulting in a maximal twofold to sixfold increase in the number of surviving tyrosine hydroxylase-IR **neurons** at various days in vitro. This increase in **dopaminergic** cell survival was not mimicked by **GDNF**, **BDNF** or NT-3 within the initial 3 days of cultivation. Moreover, initial biochemical characterization demonstrated that the neurotrophic activity is restricted to the high MW fraction of >50 kD of glial cell line-CM. Since the apparent MW of this factor exceeds the size of most known growth factors, it may represent a novel **dopaminergic** neurotrophic factor.

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(FILE 'HOME' ENTERED AT 17:06:01 ON 12 DEC 2001)

FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, BIOSIS, MEDICONF'
ENTERED AT 17:07:05 ON 12 DEC 2001
L1 22660 S MESENCEPHALON
L2 11586 S L1 AND (NEURON OR NEURAL)
L3 4156 S L2 AND (IMMORTAL? OR TRANS?)
L4 2023 S L3 AND (DOPAMINE? OR GABA?)
L5 279 S L4 AND (GDNF OR CTNF OR IGF-I OR BDNF OR EGF OR PDGF)
L6 1 S L5 AND V-MYC
L7 0 S L1 AND (V-MVC OR TERTACYCLINE)
L8 6 S L1 AND (V-MVC OR TETRACYCLINE)
L9 8 S L1 AND (V-MYC OR TETRACYCLINE)
L10 5 DUP REM L9 (3 DUPLICATES REMOVED)

=> d l10 3 all

L10 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2001 ACS
AN 2000:133811 CAPLUS
DN 132:177726
TI Human **mesencephalon** cell lines and methods of use therefor
IN Sah, Dinah W.; Raymon, Heather K.
PA Signal Pharmaceuticals, Inc., USA
SO PCT Int. Appl., 28 pp.
CODEN: PIXXD2
DT Patent
LA English
IC ICM C12N005-10
ICS A61K048-00
CC 9-11 (Biochemical Methods)
Section cross-reference(s): 1, 3, 14
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2000009669	A1	20000224	WO 1999-US18403	19990812
	W: AU, CA, JP				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	AU 9954825	A1	20000306	AU 1999-54825	19990812
	EP 1105464	A1	20010613	EP 1999-941107	19990812
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
PRAI	US 1998-134771	A	19980812		
	WO 1999-US18403	W	19990812		

AB Conditionally-immortalized human **mesencephalon** cell lines are provided. Such cell lines, which may be clonal, may be used to generate neurons, including dopaminergic neurons. The cell lines and/or differentiated cells may be used for the development of therapeutic agents to prevent and treat a variety of neurol. diseases such as Parkinson's disease. The cell lines and/or differentiated cells may also be used in assays and for the general study of **mesencephalon** cell development and differentiation.
ST immortalization **mesencephalon** culture therapy Parkinson neuron disease
IT Neurotrophic factors
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(BDNF (brain derived) and GDNF (glia-derived), cell diffentiation agent; human **mesencephalon** cell lines and methods of use therefor)
IT Tumor necrosis factors
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(CTNF, cell diffentiation agent; human **mesencephalon** cell

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lines and methods of use therefor)
IT Nervous system
(disease; human **mesencephalon** cell lines and methods of use therefor)
IT Neuron
(dopaminergic and GABA-ergic; human **mesencephalon** cell lines and methods of use therefor)
IT Cell differentiation
Cell proliferation
Embryo, animal
Genetic selection
Mammal (Mammalia)
(human **mesencephalon** cell lines and methods of use therefor)
IT Transformation, neoplastic
(immortalization; human **mesencephalon** cell lines and methods of use therefor)
IT Animal cell line
(**mesencephalon**, conditionally-immortalized human neural precursor cell; human **mesencephalon** cell lines and methods of use therefor)
IT Brain
(midbrain; human **mesencephalon** cell lines and methods of use therefor)
IT Transplant and Transplantation
(of a human **mesencephalon** cell; human **mesencephalon** cell lines and methods of use therefor)
IT Cell death
(of human **mesencephalon** neural precursor, agents causing; human **mesencephalon** cell lines and methods of use therefor)
IT Cell adhesion
(on different substrates, selection of transfected cells; human **mesencephalon** cell lines and methods of use therefor)
IT Gene, animal
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(oncogene, **v-myc**; human **mesencephalon** cell lines and methods of use therefor)
IT Proteins, specific or class
RL: BOC (Biological occurrence); BIOL (Biological study); OCCU (Occurrence)
(produced by human **mesencephalon** cells, detection of; human **mesencephalon** cell lines and methods of use therefor)
IT Transgene
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(regulatable growth-promoting; human **mesencephalon** cell lines and methods of use therefor)
IT Proteins, specific or class
RL: BOC (Biological occurrence); BIOL (Biological study); OCCU (Occurrence)
(regulating human **mesencephalon** cell death, detection of; human **mesencephalon** cell lines and methods of use therefor)
IT Parkinson's disease
(treatment of; human **mesencephalon** cell lines and methods of use therefor)
IT 66575-29-9, Forskolin 67763-96-6, IGF-1
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(cell diffentiation agent; human **mesencephalon** cell lines and methods of use therefor)
IT 60-54-8, **Tetracycline**
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(inhibition of growth-promoting gene; human **mesencephalon** cell lines and methods of use therefor)
RE.CNT 8

Print selected from Online session

RE.

(1) DI Porzio, U; SOCIETY FOR NEUROSCIENCE ABSTRACTS 1992, V18(1-2), PP410
(2) Hartikka, J; JOURNAL OF NEUROSCIENCE RESEARCH 1992, V32, P190 CAPLUS
(3) Heller, A; BRAIN RESEARCH 1996, V725(1), P132 CAPLUS
(4) Hoshimaru, M; PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES 1996, V93(4), P1518 CAPLUS
(5) Prasad, K; IN VITRO CELL DEV BIOL ANIM 1994, V30A(9), P596 MEDLINE
(6) Sah, D; NATURE BIOTECHNOLOGY 1997, V15(6), P574 CAPLUS
(7) Signal Pharm Inc; WO 9810058 A 1998 CAPLUS
(8) Zhou, J; BRAIN RESEARCH 1994, V656(1), P147 CAPLUS

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STIC-ILL

QH585.ISPT.A

From: Kaushal, Sumesh
Sent: Thursday, December 06, 2001 5:53 PM
To: STIC-ILL
Subject: ref-req#09134771

Adams FS, et al.
Characterization and transplantation of two neuronal cell lines with dopaminergic properties.
Neurochem Res. 1996 May;21(5):619-27.
PMID: 8726972; UI: 96328932.

Prasad KN, et al.
Establishment and characterization of immortalized clonal cell lines from fetal rat mesencephalic tissue.
In Vitro Cell Dev Biol Anim. 1994 Sep;30A(9):596-603.
PMID: 7820310; UI: 95120206.

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Mail Box: 12E12

4 citations found

Brain Res 2000 Jun 2;866(1-2):33-43

Overexpression of human alpha-synuclein causes dopamine neuron death in rat primary culture and immortalized mesencephalon-derived cells.

Zhou W, Hurlbert MS, Schaack J, Prasad KN, Freed CR

Division of Clinical Pharmacology C-237, Department of Medicine, and the Neuroscience Program, University of Colorado Health Sciences Center, 4200 East Ninth Avenue, Denver, CO 80262, USA.

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the appearance of intracytoplasmic inclusions called Lewy bodies (LB) in dopamine neurons in the substantia nigra and the progressive loss of these neurons. Recently, mutations in the alpha-synuclein gene have been identified in early-onset familial PD, and alpha-synuclein has been shown to be a major component of LB in all patients. Yet, the pathophysiological function of alpha-synuclein remains unknown. In this report, we have investigated the toxic effects of adenovirus-mediated alpha-synuclein overexpression on dopamine neurons in rat primary mesencephalic cultures and in a rat dopaminergic cell line - the large T-antigen immortalized, mesencephalon-derived 1RB3AN27 (N27). Adenovirus-transduced cultures showed high-level expression of alpha-synuclein within the cells. Overexpression of human mutant alpha-synuclein (Ala(53)Thr) selectively induced apoptotic programmed cell death of primary dopamine neurons as well as N27 cells. The mutant protein also potentiated the neurotoxicity of 6-hydroxydopamine (6-OHDA). By contrast, overexpression of wild-type human alpha-synuclein was not directly neurotoxic but did increase cell death after 6-OHDA. Overexpression of wild-type rat alpha-synuclein had no effect on dopamine cell survival or 6-OHDA neurotoxicity. These results indicate that overexpression of human mutant alpha-synuclein directly leads to dopamine neuron death, and overexpression of either human mutant or human wild-type alpha-synuclein renders dopamine neurons more vulnerable to neurotoxic insults.

PMID: 10825478, UI: 20286364

Mol Genet Metab 1998 Sep;65(1):1-9

Efficacy of grafted immortalized dopamine neurons in an animal model of parkinsonism: a review.

Prasad KN, Clarkson ED, La Rosa FG, Edwards-Prasad J, Freed CR

Center for Vitamins and Cancer Research, University of Colorado Health Sciences Center, Denver, Colorado 80262, USA.

Dopamine (DA) deficiency is one of the primary lesions in the pathogenesis of Parkinson disease (PD). Because of long-term toxicity of L-DOPA therapy, the grafting of fetal mesencephalic tissue containing dopamine neurons or homogeneous populations of DA neurons into striatum appears to be rational. Fetal tissue transplants have many problems which include legal (in some countries), ethical, paucity of tissue availability, heterogenicity of cell populations, and the presence of antigen-presenting cells that are responsible for rejection of allogeneic grafts. In order to resolve the above problems, we have established immortalized DA neurons from fetal rat mesencephalon by inserting the large T-antigen (LTa) gene of the SV40 virus into the cells. A clone of DA neurons (1RB3AN27) was isolated, characterized, and tested in 6-hydroxydopamine (6-OHDA)-lesioned rats (a model of PD). These cells divided with a doubling time of about 26 h, expressed the LTa gene, and contained the tyrosine hydroxylase and dopamine transporter proteins and their respective mRNAs, which became elevated upon differentiation. These cells were nontumorigenic and nonimmunogenic and improved the symptoms of neurological deficits (methamphetamine-induced rotation) in 6-OHDA-lesioned rats. The differentiated DA neurons were more effective than undifferentiated ones. These studies suggest that immortalized DA neurons generated in vitro by LTa gene insertion may be used in transplant therapy without fear of tumor formation or rejection.

Publication Types:

- Review
- Review, tutorial

PMID: 9787089, UI: 99005503

Neurochem Res 1996 May;21(5):619-27

Characterization and transplantation of two neuronal cell lines with dopaminergic properties.

Adams FS, La Rosa FG, Kumar S, Edwards-Prasad J, Kentroti S, Vernadakis A, Freed CR, Prasad KN

Department of Medicine, University of Colorado Health Sciences Center, Denver 80262, USA.

Immortalized rat mesencephalic cells (1RB3AN27) produced dopamine (DA) at a level that was higher than produced by undifferentiated or differentiated murine neuroblastoma cells (NBP2) in culture. Treatment of 1RB3AN27 and NBP2 cells with a cAMP stimulating agent increased tyrosine hydroxylase (TH) activity and the intensity of immunostaining for the DA transporter protein (DAT). 1RB3AN27 cells were labelled with primary antibodies to neuron specific enolase (NSE) and nestin and exhibited very little or no labeling with anti-glial fibrillary acidic protein (GFAP). 1RB3AN27 cells exhibited beta- and alpha-adrenoreceptors, and prostaglandin E1 receptors, all of which were linked to adenylate cyclase (AC). Dopamine receptor (D1) and cholinergic muscarinic receptors linked to AC were not detectable. The levels of PKC alpha and PKC beta isoforms were higher than those of PKC gamma and PKC delta in 1RB3AN27 cells. The 1RB3AN27 cells were more effective in reducing the rate of methamphetamine-induced turning in rats with unilateral 6-OHDA lesion of the nigrostriatal system than differentiated NBP2 cells. The grafted 1RB3AN27 were viable as determined by DiI labelling, but they did not divide and did not produce T-antigen protein; however, when these grafted cells were cultured in vitro, they resumed production of T-antigen and proliferated after the primary glia cells and neurons of host brain died due to maturation and subsequent degeneration. Examination of H&E stained sections of the grafted sites revealed no evidence of infiltration of inflammatory cells in the grafted area suggesting that these cells were not immunogenic. They also did not form tumors.

PMID: 8726972, UI: 96328932

In Vitro Cell Dev Biol Anim 1994 Sep;30A(9):596-603

Establishment and characterization of immortalized clonal cell lines from fetal rat mesencephalic tissue.

Prasad KN, Carvalho E, Kentroti S, Edwards-Prasad J, Freed C, Vernadakis A

Center for Vitamins and Cancer Research, School of Medicine, University of Colorado Health Sciences Center, Denver 80262.

This investigation reports for the first time the establishment of immortalized clones of dopamine-producing nerve cells in culture. Freshly prepared single-cell suspensions from fetal (12-day-old) rat mesencephalic tissue were transfected with plasmid vectors, pSV3neo and pSV5neo, using an electroporation technique. Cells were plated in tissue culture dishes which were precoated with a special substrate and contained modified MCDB-153 growth medium with 10% heat inactivated fetal bovine serum. The immortalized cells were selected by placing the transfected cells in a selection medium (modified MCDB-153 containing 400 micrograms/ml geneticin). The survivors showed the presence of T-antigens and were non-tumorigenic. Two cell lines, 1RB3 derived from cells transfected with pSV3neo, and 2RB5 derived from cells transfected with pSV5neo revealed only 1 to 2% tyrosine hydroxylase (TH)-positive cells. Repeated single-cell cloning of these cell lines by a standard technique failed to increase the number of TH-positive cells in any clones. Using three cycles of growth, alternating between hormone-supplemented, serum-free medium and serum-containing medium produced a cell line (1RB3A) that was very rich in TH-positive cells. The recloning of 1RB3A yielded clones some of which contained over 95% TH-positive cells. These cells produced homovanillic acid, a metabolite of dopamine, and may be useful not only for neural transplant but also for basic neurobiological studies.

PMID: 7820310, UI: 95120206

Kaushal, Sumesh

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Adams FS, et al.
Characterization and transplantation of two neuronal cell lines with dopaminergic properties.
Neurochem Res. 1996 May;21(5):619-27.
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PMID: 7820310; UI: 95120206.

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6 citations found

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Exp Neurol 2001 Aug;170(2):317-25

Long-term proliferation and dopaminergic differentiation of human mesencephalic neural precursor cells.

Storch A, Paul G, Csete M, Boehm BO, Carvey PM, Kupsch A, Schwarz J

Department of Neurology, University of Ulm Medical School, Ulm, Germany. alexander.storch@medizin.uni-ulm.de

We report on generation of dopamine neurons from long-term cultures of human fetal mesencephalic precursor cells. These CNS precursor cells were successfully expanded in vitro using the mitogens epidermal growth factor (EGF) and fibroblast growth factor-2 (FGF-2). Incubation of these cultures in 3% atmospheric oxygen resulted in higher cellular yields than room air. Following incubation in differentiation media containing interleukin (IL)-1b (IL-1b), IL-11, leukemia inhibitory factor (LIF), and glial cell line-derived neurotrophic factor (GDNF), up to 1% of the precursor cells converted into cells immunoreactive for tyrosine hydroxylase (TH), a marker for dopamine neurons. The TH immunoreactive cells exhibited morphological and functional properties characteristic of dopamine neurons in culture. These precursor cells might serve as a useful source of human dopamine neurons for studying the development and degeneration of human dopamine neurons and may further serve as a continuous, on-demand source of cells for therapeutic transplantation in patients with Parkinson's disease. Copyright 2001 Academic Press.

PMID: 11476598, UI: 21369608

Other Formats: Citation MEDLINE
Links: Experimental Neurology

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Exp Neurol 2000 Jul;164(1):209-14

Mesencephalic neural stem (progenitor) cells develop to dopaminergic neurons more strongly in dopamine-depleted striatum than in intact striatum.

Nishino H, Hida H, Takei N, Kumazaki M, Nakajima K, Baba H

Department of Physiology, Nagoya City University Medical School, Japan.

Epidermal growth factor (EGF)/fibroblast growth factor (FGF)-responsive stem (progenitor) cells from embryonic brain have self-renewing and multipotent properties and thus are good candidates for donor cells in neural transplantation. However, the survival and differentiation to mature neurons after grafting of stem cells into adult brain are rather poor. We hypothesize that the differentiation of stem cells to mature neurons, such as dopaminergic (DAergic) neurons, is dependent on environmental cues that control the ontogenic development. We compared the survival and differentiation between mesencephalic (MS) and cortical (CTx) stem (progenitor) cells, following grafting into bilateral striata of hemiparkinsonian model rats. MS and CTx stem cells were prepared from E12 rats and proliferated in serum-free medium with EGF or basic FGF for 2 weeks. One day after being primed to

differentiate, the cell suspensions of both origins were grafted into the bilateral striata of adult rats that had unilateral 6-OHDA lesions in the substantia nigra. MS cells differentiated to tyrosine hydroxylase (TH)-positive neurons more strongly in DA-depleted striatum than in intact striatum, and methamphetamine-induced rotation was ameliorated in half of the grafted animals. Rosette-like cell aggregation and dysfunction of the blood-brain barrier (BBB) were less in and around the grafts in DA-depleted striatum, suggesting less proliferation and more differentiation of MS stem cells in DA-depleted striatum. Neither TH-positive neurons nor behavioral amelioration were detected following CTx stem (progenitor) cell transplantation in the striata. Data suggest that the DA-depleted striatum offers a suitable environment for MS stem (progenitor) cells to differentiate into mature DAergic neurons. Copyright 2000 Academic Press.

PMID: 10877931, UI: 20341198

Neuroreport 1999 Jun 23;10(9):1985-92

Mouse epidermal growth factor-responsive neural precursor cells increase the survival and functional capacity of embryonic rat dopamine neurons in vitro.

Ostenfeld T, Horn P, Aardal C, Orpen I, Caldwell MA, Svendsen CN

MRC Cambridge Centre for Brain Repair, University of Cambridge, UK.

We have grown expanded populations of epidermal growth factor (EGF)-responsive mouse striatal precursor cells and subsequently co-cultured these with primary E14 rat ventral mesencephalon. The aim of these experiments was to induce dopaminergic (DA) neuronal phenotypes from the murine precursors. While no precursor cell-derived neurons were induced to express tyrosine hydroxylase (TH), there was a dramatic 30-fold increase in the survival of rat-derived TH-positive neurons in the co-cultures. The effect was not explicable solely in terms of total plating density, and was accompanied by a significantly enhanced capacity for [3H]dopamine uptake in the co-cultures compared to rat alone cultures. The present data show that, although primary rat E14 mesencephalic cells are incapable of inducing the development of DA neurons from EGF-responsive mouse neural precursor cells, such precursors will differentiate into cells capable of enhancing the survival and overall functional efficacy of primary embryonic dopamine neurons.

PMID: 10501545, UI: 99429653

Mol Cell Neurosci 1998 Jun;11(3):99-116

Incorporation and glial differentiation of mouse EGF-responsive neural progenitor cells after transplantation into the embryonic rat brain.

Winkler C, Fricker RA, Gates MA, Olsson M, Hammang JP, Carpenter MK, Bjorklund A

Wallenberg Neuroscience Center, Lund University, Lund, S-22362, Sweden.

In vitro, epidermal growth factor (EGF)-responsive neural progenitor cells exhibit multipotent properties and can differentiate into both neurons and glia. Using an in utero xenotransplantation approach we examined the developmental potential of EGF-responsive cells derived from E14 mouse ganglionic eminences, cortical primordium, and ventral mesencephalon, after injection into the E15 rat forebrain ventricle. Cell cultures were established from control mice or from mice carrying the lacZ transgene under control of the promoters for nestin, glial fibrillary acidic protein (GFAP), or myelin basic protein (MBP). The grafted cells, visualized with mouse-specific markers or staining for the reporter gene product, displayed widespread incorporation into distinct forebrain and midbrain structures and differentiated predominantly into glial cells. The patterns of incorporation of cells from all three regions were very similar without preference for the homotopic brain areas. These results suggest that EGF-responsive progenitor cells can respond to host derived environmental cues, differentiate into cells with glial-like features, and become integrated in the developing recipient brain.

PMID: 9647689, UI: 98313401

Exp Neurol 1998 Feb;149(2):411-23

Differentiation of mesencephalic progenitor cells into dopaminergic neurons by cytokines.

Ling ZD, Potter ED, Lipton JW, Carvey PM

Department of Pharmacology, Rush-Presbyterian St. Luke's Medical Center, Chicago, Illinois 60612, USA.

Rat progenitor cells from the germinal region of the fetal mesencephalon were isolated and expanded in media containing the mitogen epidermal growth factor. These cells remained mitotically active (up to 8 months), were immunoreactive for the progenitor cell marker nestin, and were readily infected with the BAG alpha retrovirus. When incubated in complete media containing serum in poly-L-lysine-coated plates, these cells spontaneously converted to neurons and glia but rarely expressed the dopamine (DA) neuron phenotype. Nineteen different cytokines were screened for their ability to induce the DA phenotype and only interleukin (IL)-1 was found to induce the expression of the DA neuron marker tyrosine hydroxylase (TH). The addition of IL-1, IL-11, leukemia inhibitory factor (LIF), and glial cell line-derived neurotrophic factor (GDNF) were found to further increase the number of TH immunoreactive (TH-ir) cells. The addition of mesencephalic membrane fragments and striatal culture-conditioned media along with the cytokine mixture induced the expression of morphologically mature TH-ir cells that were also immunoreactive for dopa-decarboxylase, the DA transporter, and DA itself. The DA neuron cell counts were approximately 20-25% of the overall cell population and 50% of the neurofilament population. Astrocytes and oligodendrocytes were also present. These data suggest that hematopoietic cytokines participate in the development of the DA neuron phenotype. Parallels between the function of hematopoietic cytokines in bone marrow and the central nervous system may exist and be useful in understanding the factors which regulate the differentiation of neurons in the brain.

PMID: 9500954, UI: 98162476

Cell Transplant 1996 Mar-Apr;5(2):179-89

Morphological differentiation of astroglial progenitor cells from EGF-responsive neurospheres in response to fetal calf serum, basic fibroblast growth factor, and retinol.

Chiang YH, Silani V, Zhou FC

Department of Anatomy, Indiana University School of Medicine, Indianapolis 46202, USA.

Procurement of multipotential neuroglial stem cells is possible with the addition of epidermal growth factor (EGF). Stem cells will differentiate into neurons and glia upon the removal of EGF from the culture medium. We have previously characterized the neuronal differentiation of stem cells derived from long-term cultured nonpassage neurospheres. In the current study, we (1) characterize the morphological differentiation of the astroglial progenitor cell from 3-mo-old neurospheres, (2) examine whether the astroglial progenitor cells from neurospheres of different brain areas exhibit different differentiation responses to the same exogenous signals, and (3) test the effects of basic fibroblast growth factor (bFGF) and retinol on differentiation. Cerebral cortex, striatum, and mesencephalon cells were obtained from Embryonic Day 14 (E-14) rat fetuses and were dissociated for the procurement of neurospheres in chemically defined medium supplemented with EGF. After 3 mo in culture, the neurospheres, derived from each of the three brain areas, were subcultured into three groups on chamber slides: (1) basal medium, (2) the basal medium plus 20 ng/mL bFGF, and (3) the basal medium plus 10 muM retinol. Phenotypic expression of astroglial cells was examined after 14 days subculture. Our findings indicate that the 3-mo-old cultured nonpassage neurospheres contained numerous multipotential stem cells that stained positive with nestin, and that environmental factors played an important role in influencing the differentiation of astroglial progenitor cells. As detected by glial fibrillary acid protein (GFAP), astroglial progenitor cells turned into protoplasmic astrocytes in the FCS-containing basal medium, fibrous astrocytes in the presence of bFGF, and spindle-shaped astrocytes in the presence of retinol. There were no noticeable differences in differentiation among astroglial progenitor cells of the various brain region-derived neurospheres in any of the three medium conditions. Peculiar varicosity-and growth cone-like structures on the long slender GFAP-positive processes suggest that neuroblasts and glioblast may share common morphologies, features, or common progenitor cells during initial differentiation in vitro.

PMID: 8689030, UI: 96254798

5 citations found

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Exp Neurol 1996 Feb;137(2):376-88

Survival and differentiation of rat and human epidermal growth factor-responsive precursor cells following grafting into the lesioned adult central nervous system.

Svendsen CN, Clarke DJ, Rosser AE, Dunnett SB

MRC Cambridge Centre for Brain Repair, University of Cambridge, United Kingdom.

Epidermal Growth Factor (EGF)-responsive stem cells isolated from the developing central nervous system (CNS) can be expanded exponentially in culture while retaining the ability to differentiate into neurons and glia. As such, they represent a possible source of tissue for neural transplantation, providing they can survive and mature following grafting into the adult brain. In this study we have shown that purified rat stem cells generated from either the embryonic mesencephalon or the striatum can survive grafting into the striatum of rats with either ibotenic acid or nigrostriatal dopamine lesions. However, transplanted stem cells do not survive as a large mass typical of primary embryonic CNS tissue grafts, but in contrast form thin grafts containing only a small number of surviving cells. There was no extensive migration of transplanted stem cells labeled with either the lac-z gene or bromodeoxyuridine into the host region surrounding the graft, although a small number of labeled cells were seen in the ventral striatum some distance from the site of implantation. Some of these appeared to differentiate into dopamine neurons, particularly when the developing mesencephalon was used as the starting material for generating the stem cells. EGF-responsive stem cells could also be isolated from the mesencephalon of developing human embryos and expanded in culture, but only grew in large numbers when the gestational age of the embryo was greater than 11 weeks. Purified human CNS stem cells were also transplanted into immunosuppressed rats with nigrostriatal lesions and formed thin grafts similar to those seen when using rat stem cells. However, when primary cultures of human mesencephalon were grown with EGF for only 10 days and this mixture of stem cells and primary neural tissue was transplanted into the dopamine-depleted striatum, large well-formed grafts developed. These contained mostly small undifferentiated cells intermixed with a number of well-differentiated TH-positive neurons. These results show that purified populations of rat or human EGF-responsive CNS stem cells do not form large graft masses or migrate extensively into the surrounding host tissues when transplanted into the adult striatum. However, modifications of the growth conditions in vitro may lead to an improvement of their survival in vivo.

PMID: 8635554, UI: 96178061

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J Neurosci Res 1995 Oct 1;42(2):172-83

Epidermal growth factor (EGF), transforming growth factor-alpha (TGF-alpha), and basic fibroblast growth factor (bFGF) differentially

influence neural precursor cells of mouse embryonic mesencephalon.

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Growth factors are key elements in the process of neural cell differentiation. We examined the effects of classical mitogens on neural precursor cells, by culturing mouse cells of the embryonic (13.5 days postcoitum) mesencephalon and treating them with epidermal growth factor (EGF), transforming growth factor-alpha (TGF-alpha), basic fibroblast growth factor (bFGF), nerve growth factor (NGF), and transforming growth factor-beta (TGF-beta). Our initial results show that EGF, TGF-alpha, or bFGF, but not NGF or TGF-beta, induced general proliferation of the cultured cells, followed by formation of colonies. Combinations of these three growth factors suggest that most cells with the capacity to form colonies responded to EGF, TGF-alpha, or bFGF. The number of colonies increased significantly when EGF, but not TGF-alpha, was used in combination with bFGF. Furthermore, a population responding only to EGF + bFGF was detected in the dorsal mesencephalon. The colony-forming activity of bFGF was dependent on insulin, but bFGF and insulin cooperation was indirect since we could not observe colony formation in subcultures of cells derived from colonies, even in the presence of insulin. Cells obtained from our colonies displayed neuronal and glial morphology and expressed markers of both neurons and astrocytes; nestin, a marker of neural precursor cells, was also expressed in the majority of colonies. Growth factors also influenced neuronal maturation; the best neurite outgrowth was obtained from cells derived from bFGF-induced colonies cultured in the presence of EGF + bFGF. These data indicate the existence of neural precursor cells in the embryonic mesencephalon that respond differentially to growth factors.

PMID: 8568917, UI: 96122596

Exp Brain Res 1995;102(3):407-14

Increased survival of rat EGF-generated CNS precursor cells using B27 supplemented medium.

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Previous studies suggest that a population of precursor cells from the developing and adult mouse striatum can be expanded in culture using serum-free, N2-supplemented medium and mitogenic factors such as epidermal growth factor (EGF). Here we show that EGF-responsive precursor cells from embryonic rat striatum and mesencephalon can also be expanded in culture, incorporate bromodeoxy uridine (BrDU) and develop into spheres that either adhere to the surface of the culture dish or float freely in the medium. Addition of B27, a medium supplement that increases neuronal survival in primary CNS cultures, resulted in a tenfold increase in the number of proliferating cells in vitro over the first week. The effects of B27-supplemented medium on precursor cell survival were only seen when primary cultures were used, such that dividing cells grown in B27 for 1 week could then be transferred to either B27 or N2 medium and show similar survival and division rates in response to EGF. After 1, 2 or 4 weeks of growth in B27-supplemented medium, dissociated precursor cells from either striatal or mesencephalic cultures could be differentiated when exposed to a poly-l-lysine-coated substrate in serum and EGF-free medium supplemented with B27. These cells then matured into a mixed culture containing neurons (approximately 35% of cells), astrocytes (approximately 44% of cells), and oligodendrocytes (approximately 10% of cells), based on immunocytochemical staining with microtuble-associated protein (MAP2), glial fibriallary acidic protein and galactocerebrosidase. When whole spheres of precursor cells were allowed to differentiate, every one examined was found to generate neurons, astrocytes and oligodendrocytes in similar proportions.

PMID: 7737387, UI: 95255531

J Neurochem 1994 Jun;62(6):2166-77

Epidermal growth factor and basic fibroblast growth factor have independent actions on mesencephalic dopamine neurons in culture.

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Epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) are both trophic for dopamine neurons in cultures of dissociated embryonic rat mesencephalon, but the significance of this apparent overlap in neurotrophic activity is not yet known. In this study, we investigated the mechanisms of action of these two growth factors and the potential relationship between them. Using a nuclease protection assay, we determined that bFGF mRNA was expressed in the cultures. Double-label immunocytochemistry revealed that bFGF immunoreactive material could be detected in tyrosine hydroxylase immunoreactive neurons and glial fibrillary acidic protein immunoreactive astrocytes. EGF treatment increased bFGF mRNA content per culture dish. As we have previously demonstrated that EGF exerts its dopaminergic neurotrophic activity via an intermediate cell type, studies were designed to address whether the pathway by which EGF acts on dopaminergic neurons is mediated by the release of bFGF. However, the trophic action of EGF on dopamine neurons, represented by high-affinity neuronal dopamine uptake, could not be blocked by immunoneutralization of bFGF, suggesting that the actions of EGF were not mediated by bFGF release. The time course of the effects of EGF and bFGF on dopamine uptake were similar, with significant increases detectable only after 5 days in culture. Both growth factors were active in the picomolar-to-nanomolar range with maximal trophic activity between 0.4 and 2.5 nM. EGF, however, was the more potent mitogen under these conditions. When cultures were simultaneously incubated with maximal concentrations of EGF and bFGF, the effect on dopamine uptake was significantly greater than with either growth factor alone and, in fact, approximated the sum of the individual effects. On the basis of these results we conclude that these growth factors have independent effects on dopamine neurons of the mesencephalon.

PMID: 8189225, UI: 94246388

Exp Brain Res 1993;92(3):516-23

Platelet-derived growth factor promotes survival of rat and human mesencephalic dopaminergic neurons in culture.

Nikkhah G, Odin P, Smits A, Tingstrom A, Othberg A, Brundin P, Funa K, Lindvall O

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The effect of two isoforms of platelet-derived growth factor (PDGF), PDGF-AA and PDGF-BB, was tested on dissociated cell cultures of ventral mesencephalon from rat and human embryos. PDGF-BB but not PDGF-AA reduced the progressive loss of tyrosine hydroxylase- (TH)-positive neurons in rat and human cell cultures. The mean number of TH-positive cells in the PDGF-BB-treated rat culture was 64% and 106% higher than in the control

cultures after 7 and 10 days in vitro, respectively. Corresponding figures for human TH-positive neurons were 90% and 145%. The influence of PDGF-BB was specific for TH-positive neurons and not a general trophic effect, since no change of either total cell number or metabolic activity was found. In PDGF-BB-treated cultures of human but not rat tissue the TH-positive neurons had longer neurites than observed in control or PDGF-AA-treated cultures. These data indicate that PDGF-BB may act as a trophic factor for mesencephalic dopaminergic neurons and suggest that administration of PDGF-BB could ameliorate degeneration and possibly promote axonal sprouting of these neurons in vivo.

PMID: 8095907, UI: 93202215

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Neurosci Lett 1992 Jan 20;135(1):62-6

Epidermal growth factor-induced survival and proliferation of neuronal precursor cells from embryonic rat mesencephalon.

Mytilineou C, Park TH, Shen J

Department of Neurology, Mount Sinai School of Medicine, New York, NY 10029.

Neuronal and glial precursor cells were isolated from primary cultures of embryonic rat mesencephalon. The separation of precursor cells from the neurons was accomplished by the resuspension of the primary cells by trypsinization, followed by replating. This procedure resulted in the death of differentiated neurons and the survival of precursor cells. The survival and proliferation of the replated precursor cells required the presence of epidermal growth factor (EGF) in the culture medium. The precursor cells differentiated into neurons and astrocytes, as determined by immunocytochemical staining with antibodies to neuron specific enolase (NSE) and tau protein or glial fibrillary acidic protein (GFAP) respectively.

PMID: 1542439, UI: 92178670

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J Neurosci Res 1991 Nov;30(3):493-7

Epidermal growth factor exerts neuronotrophic effects on dopaminergic and GABAergic CNS neurons: comparison with basic fibroblast growth factor.

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Basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF) have been described to exert neuronotrophic effects on central nervous system neurons in culture. To study the selectivity of trophic actions of these growth factors, neurotransmitter-identified populations of embryonic rat mesencephalon were used. At 20 days in vitro, EGF (3 ng/ml) promoted survival and neurite outgrowth from these neurons. The neuritogenic effect of bFGF (3 ng/ml) was, however, more robust. Quantitative analysis with the neurofilament monoclonal antibody RR97 and ELISA confirmed the differential response, bFGF being 2-2.5 times more effective at all concentrations tested (ED100: 3-10 ng/ml for both EGF and bFGF). At 10 days in vitro, EGF displayed no trophic activity--even at 30 ng/ml. Treatment of mesencephalic cultures with EGF (3 ng/ml) for 20 days stimulated [3H]dopamine and [14C]GABA uptakes about 4-fold. While bFGF (3 ng/ml) also stimulated GABA uptake some 4-fold, dopamine uptake was increased almost 20-fold. Thus, EGF is also capable of enhancing the transmitter traits of selected central neuronal populations; however, the actions of bFGF appear to preferentially address dopaminergic cells.

PMID: 1800771, UI: 92194352

J Neurosci Res 1991 Oct;30(2):372-81

EGF enhances the survival of dopamine neurons in rat embryonic mesencephalon primary cell culture.

Casper D, Mytilineou C, Blum M

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Epidermal growth factor (EGF) immunoreactive material has been demonstrated to be present in the basal ganglia. In this study, we investigated the effect of EGF on cells cultured from 16-day embryonic rat mesencephalon, which included dopamine neurons that project to the striatum in vivo. EGF receptors were detected in untreated cultures by [125I]-EGF binding. Treatment of the cultures with EGF resulted in up to 50-fold increases in neuronal high-affinity dopamine uptake. Scatchard analysis of uptake kinetics and counting of tyrosine hydroxylase-immunoreactive cells suggest that the effect of EGF on uptake is due to increased survival and maturation of dopaminergic neurons. By contrast, the high-affinity uptake for serotonin was increased only threefold over its controls. There was no significant effect on high-affinity gamma-aminobutyric acid (GABA) uptake. These results suggest that EGF is acting as a neurotrophic agent preferential for dopaminergic neurons in E16 mesencephalic cultures. Immunocytochemistry for glial fibrillary acidic protein demonstrated an increase in astroglia with EGF treatment. Fluorodeoxyuridine, an agent that is toxic to proliferating cells was able to eliminate the effect of EGF on dopamine uptake, suggesting that EGF may be increasing dopaminergic cell survival largely through a population of dividing cells.

PMID: 1839162, UI: 92185921

Cell Transplant 1996 Mar-Apr;5(2):179-89

Morphological differentiation of astroglial progenitor cells from EGF-responsive neurospheres in response to fetal calf serum, basic fibroblast growth factor, and retinol.

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Procurement of multipotential neuroglial stem cells is possible with the addition of epidermal growth factor (EGF). Stem cells will differentiate into neurons and glia upon the removal of EGF from the culture medium. We have previously characterized the neuronal differentiation of stem cells derived from long-term cultured nonpassage neurospheres. In the current study, we (1) characterize the morphological differentiation of the astroglial progenitor cell from 3-mo-old neurospheres, (2) examine whether the astroglial progenitor cells from neurospheres of different brain areas exhibit different differentiation responses to the same exogenous signals, and (3) test the effects of basic fibroblast growth factor (bFGF) and retinol on differentiation. Cerebral cortex, striatum, and mesencephalon cells were obtained from Embryonic Day 14 (E-14) rat fetuses and were dissociated for the procurement of neurospheres in chemically defined medium supplemented with EGF. After 3 mo in culture, the neurospheres, derived from each of the three brain areas, were subcultured into three groups on chamber slides: (1) basal medium, (2) the basal medium plus 20 ng/mL bFGF, and (3) the basal medium plus 10 muM retinol. Phenotypic expression of astroglial cells was examined after 14 days subculture. Our findings indicate that the 3-mo-old cultured nonpassage neurospheres contained numerous multipotential stem cells that stained positive with nestin, and that environmental factors played an important role in influencing the differentiation of astroglial progenitor cells. As detected by glial fibrillary acid protein (GFAP), astroglial progenitor cells turned into protoplasmic astrocytes in the FCS-containing basal medium, fibrous astrocytes in the presence of bFGF, and spindle-shaped astrocytes in the presence of retinol. There were no noticeable differences in differentiation among astroglial progenitor cells of the various brain region-derived neurospheres in any of the three medium conditions. Peculiar varicosity-and growth cone-like structures on the long slender GFAP-positive processes suggest that neuroblasts and glioblast may share common morphologies, features, or common progenitor cells during initial differentiation in vitro.

PMID: 8689030, UI: 96254798

Exp Neurol 2001 Aug;170(2):317-25

Long-term proliferation and dopaminergic differentiation of human mesencephalic neural precursor cells.

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We report on generation of dopamine neurons from long-term cultures of human fetal mesencephalic precursor cells. These CNS precursor cells were successfully expanded in vitro using the mitogens epidermal growth factor (EGF) and fibroblast growth factor-2 (FGF-2). Incubation of these cultures in 3% atmospheric oxygen resulted in higher cellular yields than room air. Following incubation in differentiation media containing interleukin (IL)-1b (IL-1b), IL-11, leukemia inhibitory factor (LIF), and glial cell line-derived neurotrophic factor (GDNF), up to 1% of the precursor cells converted into cells immunoreactive for tyrosine hydroxylase (TH), a marker for dopamine neurons. The TH immunoreactive cells exhibited morphological and functional properties characteristic of dopamine neurons in culture. These precursor cells might serve as a useful source of human dopamine neurons for studying the development and degeneration of human dopamine neurons and may further serve as a continuous, on-demand source of cells for therapeutic transplantation in patients with Parkinson's disease. Copyright 2001 Academic Press.

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Brain Res Dev Brain Res 1996 May 31;93(1-2):10-7

Proteins from chromaffin granules promote survival of dorsal root ganglionic neurons: comparison with neurotrophins.

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Neurotrophins are established survival and differentiation factors for sensory dorsal root ganglionic (DRG) neurons. We have previously shown that proteins from the secretory granules of adrenal chromaffin cells have a capacity to promote the survival of cultured chick DRG neurons. Using DRG neurons from embryonic day (E) 8 chick embryos we show now that this material is (i) as effective as nerve growth factor (NGF), (ii) additive to NGF, neurotrophin-3, or -4, (iii) unlikely to be a neurotrophin, since the survival promoting effect can not be blocked by K252b, a specific inhibitor of the signal transduction pathways of neurotrophin high affinity receptors, (iv) partially blockable by antibodies to transforming growth factor-beta (TGF-beta) 1/2/3, and (v) more potent than any other out of 30 cytokines tested individually, including fibroblast growth factor (FGF)-5, epidermal growth factor (EGF), TGF-alpha, platelet-derived growth factor (PDGF)-AB, insulin-like growth factors (IGF)-I and -II, leukemia inhibitory factor (LIF), TGF-beta, glial cell line-derived neurotrophic factor (GDNF), stem cell factor, granulocyte-colony stimulating factor (G-CSF), oncostatin M, tumor necrosis factor (TNF)-alpha, and interleukins (IL)-1 through -12. We conclude that chromaffin cells, which are known to receive a sensory innervation, can provide (a) trophic factor(s), which, in addition to neurotrophins, may be relevant for the maintenance of DRG neurons.

PMID: 8804687, UI: 96397794

Mol Biol Cell 1992 May;3(5):545-53

The beta-PDGF receptor induces neuronal differentiation of PC12 cells.

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Expression of the mouse beta-PDGF receptor by gene transfer confers PDGF-dependent and reversible neuronal differentiation of PC12 pheochromocytoma cells similar to that observed in response to NGF and basic FGF. A common property of the PDGF, NGF, and basic FGF-induced differentiation response is the requirement for constant exposure of cells to the growth factor. To test the hypothesis that a persistent level of growth factor receptor signaling is required for the maintenance of the neuronal phenotype, we examined the regulation of the serine/threonine-specific MAP kinases after either short- (10 min) or long-term (24 h) stimulation with growth factors. Mono Q FPLC resolved two peaks of growth factor-stimulated MAP kinase activity that coeluted with tyrosine phosphorylated 41- and 43-kDa polypeptides. MAP kinase activity was markedly stimulated (approximately 30-fold) within 5 min of exposure to several growth factors (PDGF, NGF, basic FGF, EGF, and IGF-I), but was persistently maintained at 10-fold above basal activity after 24 h only by the growth factors that also induce PC12 cell differentiation (PDGF, NGF, and basic FGF). Thus the beta-PDGF receptor is in a subset of tyrosine kinase-encoded growth factor receptors that are capable of maintaining continuous signals required for differentiation of PC12 cells. These signals include the constitutive activation of cytoplasmic serine/threonine protein kinases.

PMID: 1319243, UI: 92305425

Dev Biol 1996 Apr 10;175(1):1-13

Clonal and population analyses demonstrate that an EGF-responsive mammalian embryonic CNS precursor is a stem cell.

Reynolds BA, Weiss S

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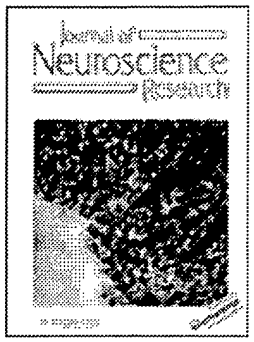
In cultures of embryonic striatum, we previously reported that EGF induces the proliferation of single precursor cells, which give rise to spheres of undifferentiated cells that can generate neurons and glia. We report here that, in vitro, these embryonic precursor cells exhibit properties and satisfy criteria representative of stem cells. The EGF-responsive cell was able to generate the three major phenotypes of the mammalian CNS--neurons, astrocytes, and oligodendrocytes. Approximately 90% of both primary spheres and secondary expanded clones, derived from the primary spheres, contained all three cell types. The increase in frequency of EGF-generated spheres, from 1% in primary culture to close to 20% in secondary culture, and the large number of clonally derived secondary spheres that could be generated from a single primary sphere indicate that EGF induces both renewal and expansion of the precursor cell itself. In population studies, the EGF-responsive cells were carried through 10 passages, resulting in a 10(7)-fold increase in cell number, without losing their proliferative and multilineage potential. Thus, this study describes the first demonstration, through clonal and population analyses in vitro, of a mammalian CNS stem cell that proliferates in response to an identified growth factor (EGF) and produces the three principal cell types of the CNS.

PMID: 8608856, UI: 96184937

Article Abstract



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Evidence for a novel neurotrophic factor for dopaminergic neurons secreted from mesencephalic glial cell lines

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Keywords

catecholamines; neurotropism; tyrosine hydroxylase; O-2A progenitor cells; glial fibrillary acidic protein; astrocytes; neurotrophins; GDNF

Abstract

Our previous studies have shown that primary mesencephalic glia secrete factors that promote dopaminergic cell survival and differentiation in vitro. To obtain enough starting material to identify the neurotrophic activity, embryonic day (E)14.5 rat mesencephalic glia were stimulated with acidic fibroblast growth factor to increase number of cells. These cells were replated in the absence of neurons and immortalized by transfection with the SV 40 large T-antigen. Clonal cell lines were established and characterized for immunoreactivity (IR) to various glial and non-glial markers. Media conditioned by these cell lines were tested for survival-promoting effects on dopaminergic neurons in serum-free cultures of the dissociated E14.5 rat mesencephalon. All cell lines expressed IR for the astrocytic marker, GFAP, the oligodendroglial marker, CNP, and for A2B5, a marker for O-2A progenitor cells, but were negative for the neuronal marker, microtubule associated protein-2, and the fibroblast marker, fibronectin. Moreover, treatment of serum-free cultures of the dissociated E14.5 mesencephalon with glial cell line-conditioned medium (CM) delayed dopaminergic cell death in a dose-dependent manner, resulting in a maximal twofold to sixfold increase in the number of surviving tyrosine hydroxylase-IR neurons at various days in vitro. This increase in dopaminergic cell survival was not mimicked by GDNF, BDNF or NT-3 within the initial 3 days of cultivation. Moreover, initial biochemical characterization demonstrated that the neurotrophic activity is restricted to the high MW fraction of >50 kD of glial cell line-CM. Since the apparent MW of this factor exceeds the size of most known growth factors, it may represent a novel dopaminergic neurotrophic factor. © 1996 Wiley-Liss, Inc.

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bFGF regulates the proliferative fate of unipotent (neuronal) and bipotent (neuronal/astroglial) EGF-generated CNS progenitor cells.

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In cultures of embryonic and adult mouse striatum, we previously demonstrated that EGF induces the proliferation of putative stem cells, which give rise to spheres of undifferentiated cells that can generate neurons and astrocytes. We report here that the spheres of undifferentiated cells contain mRNA and protein for the FGF receptor (FGFR1). Indirect immunocytochemistry demonstrated that many of the cells within the EGF-generated spheres were immunoreactive for FGFR1. Exogenous application of bFGF to the EGF-generated cells induced the proliferation of two progenitor cell types. The first, a bipotent progenitor cell, gave rise to cells with the antigenic and morphological properties of neurons and astrocytes; the other gave rise to cells with neuronal characteristics only. bFGF-generated cells with neuronal morphology exhibited electrophysiological properties indicative of immature central neurons. These results support the hypothesis that sequential actions of growth factors play a role in regulating the generation of neurons and astrocytes in the developing CNS.

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